ORIGINAL ARTICLE

Comprehensive Gas Chromatography-Mass Spectrometry Profiling of Bioactive Compounds in Various Crude Extracts of *Eryngium Foetidum* Linn

Shaik Chand Basha[1], S. V. Satyanarayana[2]

[1]Department of Pharmaceutical Chemistry, Prabhath Institute of Pharmacy, Nandyal and Research Scholar, Jawaharlal Nehru Technological University Anantapur, Anantapuramu, Andhra Pradesh, India, [2]Department of Chemical Engineering, Jawaharlal Nehru Technological University, Anantapur, Anantapuramu, Andhra Pradesh, India

Abstract

Background: The potency and bioactive phytochemicals of the Indian miracle plant *Eryngium foetidum* Linn. were examined for the 1st time. This fragrant and therapeutic herb is utilized as a traditional food spice and in ethnomedicine. **Objective:** The objective of this study was to examine and evaluate the chemical composition of various crude extracts derived from the complete *E. foetidum* Linn. plant, which is native to India and the Philippines. **Methods:** The entire air-dried plant was ground into a powder and then, using a series of solvents with increasing polarity by Soxhlet extraction, four separate extracts were obtained: pet ether, chloroform, ethyl acetate, and ethanol. Following that, a gas chromatography-mass spectrometry (GC-MS) study was performed on each of the extracts. **Results:** Qualitative identification of several physiologically active substances in crude *E. foetidum* Linn extracts. GC-MS analysis of *E. foetidum* showed that various high and low-molecular-weight chemical entities were present in varying quantities in each of the extracts. These substances are thought to be significant from a biological and pharmacological standpoint. Moreover, each of the four extracts has distinct physicochemical properties that can be linked to the chemicals that are naturally occurring in large amounts in *E. foetidum* Linn. **Conclusion:** Major bioactive chemicals found in all four extracts have been identified and characterized using spectroscopy. Hence, additional biological and pharmacological study is necessary in light of the identification of various physiologically active compounds within the extracts of *E. foetidum.*

Key words: Bioactive phytochemicals, gas chromatography-mass spectrometry analysis, *Eryngium Foetidum Linn.*

INTRODUCTION

For millennia, medicinal plants have held a pivotal role in safeguarding human well-being and augmenting the human experience. Within these botanical sources lie potent pharmacological agents, encompassing essential minerals and trace elements.[1] At present, as per the World Health Organization's approximations, a substantial 70–80% of the global population continues to pivot toward unconventional therapeutic modalities, prominently hinging upon botanical derivates.[2] Among such landscapes of herbal utilization, India stands renowned for its opulent heritage of herbal medicine, with its inhabitants deeply attuned to the curative and aromatic attributes exhibited by numerous botanical specimens. Nevertheless, the intrinsic toxicity inherent to these herbal entities necessitates vigilant scrutiny, primarily stemming from potential contaminants encompassing pesticides, microorganisms, heavy metals, synthetic toxins, and adulterating agents.[3] In a broader context, the intricate interplay of factors such as geographical parameters, geochemical attributes of

Address for correspondence:
Shaik Chand Basha, Prabhath Institute of Pharmacy, Nandyal and Research Scholar, Jawaharlal Nehru Technological University Anantapur, Anantapur, Andhra Pradesh, India.
E-mail: schandbasha20@gmail.com

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the soil, existing pollutants within the soil, water, and air matrices, alongside variables governing growth, transit, and storage, collectively exerts a profound influence upon the characteristics and overall quality of herbal organisms and their constituent elements.[4]

METHODS

Plant materials

In December, the entire *Eryngium foetidum* Linn. plant was gathered from Marthandam, Kanyakumari District, Tamil Nadu. Mature, healthy plants were chosen for the investigation. Dr. C. Madhava Chetty, a professor in the botany department of Tirupati's SV University, Chittoor District, AP, India, verified the taxonomic identification of the plant and had it deposited as a sample in the herbarium. To remove the dust and debris, the plants were carefully cleaned with tap water and then again with distilled water.

Preparation of plant extracts

After being washed, the plants were divided into little pieces and left to dry in the shadows for 15 days. Before the extraction procedure, within a hot air oven, they were dried for 2 h at 40°C to eliminate the moisture balance. The Soxhlet extraction technique was used to carry out the extraction.[5] To obtain a uniformly sized powder, whole *E. foetidum* Linn. plants were ground into a fine powder with a grinding machine and then passed through an 80 mesh sieve. A thimble of Whatmann's filter paper held around 10 g of the powder. After that, the equipment was put together, and extraction was done utilizing 250 mL of petroleum ether, chloroform, ethanol, and ethyl acetate as the solvents. A constant temperature of 35–40°C was maintained. Chloroform, ethanol, and petroleum ether were extracted for 5 h, and 10 h for ethanol, according to the color of the solvent as it gathered in the thimble chamber. For phytochemical screening, Concentrated extract in 10 mL/person was kept in storage, and the leftover extracts were dried. The dried extract was employed to separate the phytoconstituents and investigate their pharmacological properties.[6]

Gas chromatography-mass spectroscopy (GC-MS) analysis of crude extract

The analysis of selected fractions was performed using the Clarus 680 (Perkin Elmer, U. S. A.) GC-MS, because the GC interfaced to an MS (GC-MS) method was a direct and fast analytical approach for the identification and characterization of biologically active principles of a complex organic and biochemical mixture based on their relative abundance, or m/z ratio.[7]

Sample preparation for GC-MS analysis

Four milliliters of solvent for every sample extract, containing 5 mg/mL, were extracted individually using a solvent that was relatively polar to the test extract, such as acetonitrile or methanol. The samples were then sonicated for 1 h and allowed to stand for about 12 h before being subjected to GC-MS analysis. Following filtration via a 0.25 µM PTFE filter, 2 mL of the final sample solution was injected into the GC-MS apparatus at a split ratio of 10:1 for analysis.[8]

Instrumentation specification for GC-MS

A Clarus 680 GC (Perkin Elmer, U.S.A.) connected to a mass spectrometer (GC-MS) equipped with an Elite 5 MS fused silica capillary column (30 m length X 0.25 mm film thickness X 250 µm df) loaded with 5% biphenyl and 95% dimethylpolysiloxane was used to analyze the crude extracts.[9] For GC-MS detection, an electron impact ionization energy system with an ionization energy of 70 eV was used. During the chromatographic run, the split ratio used was 10:1, the injection volume was 1 µL, the injector temperature was 260°C, and the ion source temperature was 230°C. The carrier gas utilized in the experiment was 99.999% helium gas. An oven temperature was set to begin with 60°C (isothermal for a duration of 120 s) and increase by 10°C each minute to 300°C, holding the temperature for 6 min. Mass spectra were obtained with 70 eV, with a 40–600 Da scan range and a 0.5 s scan interval. The GC ran for 32 min overall.[10] The relative percentage quantity was ascertained by comparing each component's average peak area to the overall areas. Turbomass version 5.4.2 was the software that was modified to handle chromatograms and mass spectra to identify and characterize unidentified phyto-components.[11]

Data acquisition and processing

The GC-MS mass spectrum was interpreted using the National Institute of Standards and Technology (NIST) 2008 database, which has over 62000 patterns. It was determined to compare the spectra of the known and unknown components.[12,13]

RESULTS

A GC-MS analysis was carried out for the crude extracts of *E. foetidum* Linn., which has shown the following results [Figures 1-4 and Tables 1-4].

DISCUSSION

E. foetidium Linn's entire plant was subjected to extraction using solvents with distinct polarities: petroleum ether,

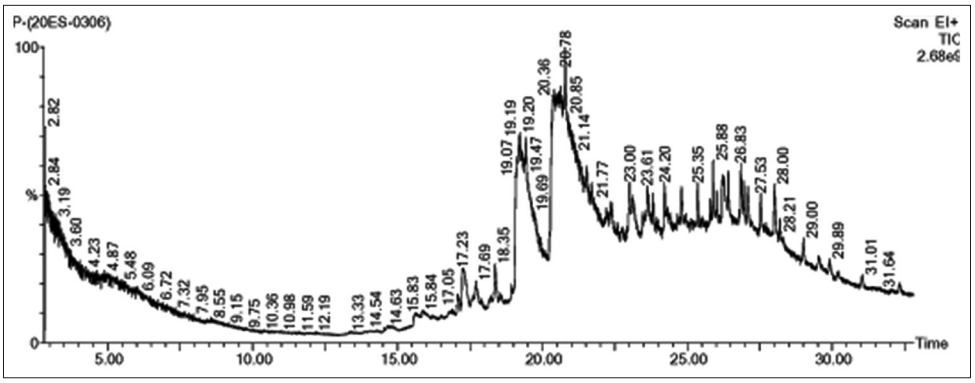

Figure 1: Chromatogram obtained from the gas chromatography-mass spectrometry of *Eryngium foetidum* Linn. Pet. ether extract

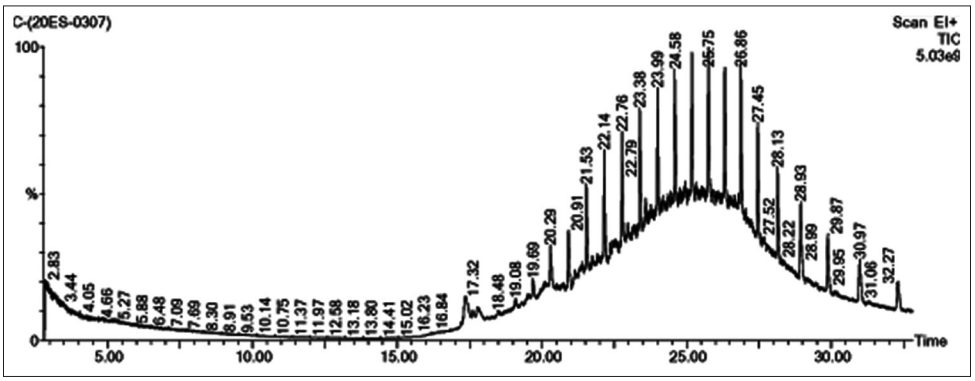

Figure 2: Chromatogram obtained from the gas chromatography-mass spectrometry of *Eryngium foetidum* Linn. chloroform extract

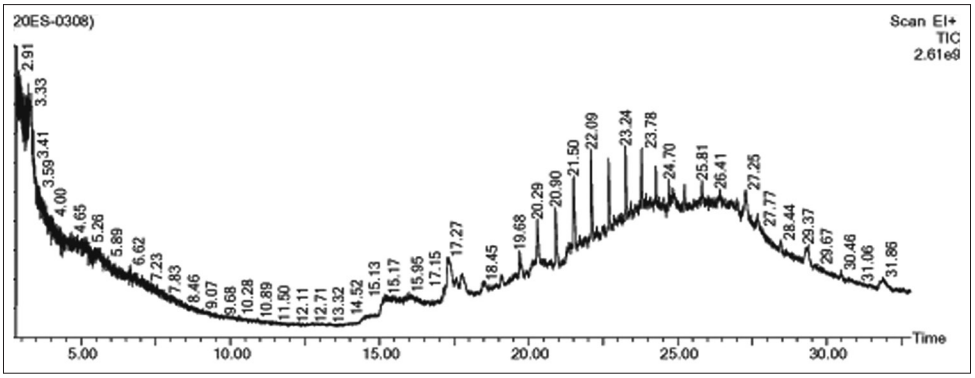

Figure 3: Chromatogram obtained from the gas chromatography-mass spectrometry of *Eryngium foetidum* Linn. ethyl acetate extract

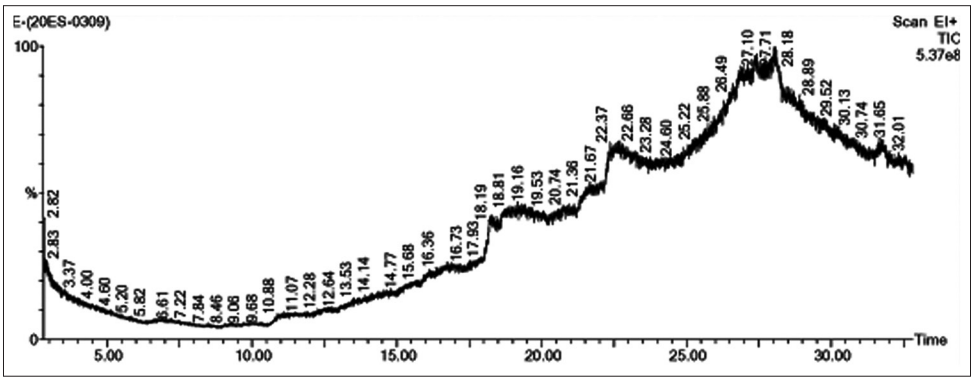

Figure 4: Chromatogram obtained from the gas chromatography-mass spectrometry of *Eryngium foetidum* Linn. ethanol extract

Table 1: GC-MS analysis of *Eryngium foetidum* Linn. Pet. Ether extract

S. No	Compound	Retention time (Min)	Area of peak (%)	Molecular formula	Molecular mass
1	9,12- octadecadien-ol	20.776	26.406	$C_{18}H_{34}O$	266
2	n- hexadecanoic acid	19,420	6.938	$C_{16}H_{32}O_2$	256
3	n-hexadecanoic acid	19,205	5.634	$C_{16}H_{32}O_2$	256
4	Octadecanoic acid, 2-oxo-methyl ester.	25.352	4.272	$C_{19}H_{36}O_3$	312

GC-MS: Gas chromatography-mass spectrometry

Table 2: GC-MS analysis of *Eryngium foetidum* Linn.Pet. chloroform extract

S. No	Compound	Retention time (Min)	Area of peak (%)	Molecular formula	Molecular mass
1	Pentatriacontane	25.753	19.226	$C_{35}H_{72}$	492
2	Sulphurous acid, butyl heptadecyl ester	26.318	11.830	$C_{21}H_{44}O_3S$	376
3	Tritetracontane	24.582	11.103	$C_{43}H_{88}$	604
4	Heptacosane	26.863	10.911	$C_{27}H_{56}$	380

GC-MS: Gas chromatography-mass spectrometry

Table 3: GC-MS analysis of *Eryngium foetidum* Linn. Pet. ethyl acetate extract

S. No	Compound	Retention time (Min)	Area of peak (%)	Molecular formula	Molecular mass
1	Tetrapentacontane, 1,54-dibromo	26.413	20.208	$C_{54}H_{108}Br_2$	914
2	Docosane, 1,22-dibromo	25.808	12.277	$C_{22}H_{44}Br_2$	466
3	Triacontane, 1,30-dibromo	27.668	8.452	$C_{30}H_{60}Br_2$	578
4	Octadecanoic acid, 2-oxo-methyl ester	27.263	7.837	$C_{19}H_{36}O_3$	312

GC-MS: Gas chromatography-mass spectrometry

Table 4: GC-MS analysis of *Eryngium foetidum* Linn. Pet. Ethanol extract

S. No	Compound	Retention time (Min)	Area of peak (%)	Molecular formula	Molecular mass
1	1-bromoeicosane	27.433	45.572	$C_{20}H_{41}Br$	360
2	Heneicosane, 11-cyclopentyl	28.033	25.400	$C_{26}H_{52}$	364
3	11-eicosenoic acid, trimethyl ester	22.686	16.715	$C_{23}H_{44}O_2Si$	382
4	5-methyl-5-phenyl-2-oxazoline	23.727	4.041	$C_{12}H_{15}ON$	189

GC-MS: Gas chromatography-mass spectrometry

chloroform, ethyl acetate, and ethanol. Each extract underwent an initial qualitative assessment to gauge preliminary phytochemical attributes, followed by confirmation of unidentified secondary metabolites using GC-MS analysis. This analysis unveiled a repertoire of 16 chemically diverse and abundant metabolites. Noteworthy instances include the identification of 9,12-octadecadien-ol in the petroleum ether extract, Pentatriacontane in the chloroform extract, tetrapentacontane, 1,54-dibromo in the ethyl acetate extract, as well as 1-bromoeicosane in the ethanolic extract, all designated as principal bioactive metabolites based on GC-MS results.

However, the current limitations of plant metabolomics hinder phytochemical analysis with GC-MS due to issues with non-volatile or derivatized compounds. To bridge this gap, we have endeavored to characterize the primary volatile metabolites of *E. foetidium* Linn. Utilizing meticulously ideal chromatographic circumstances. The reliability and consistency of the mass spectral data fragment obtained through GC-MS align exceptionally well with the NIST database.[10]

Chromatograms generated from diverse extracts of *E. foetidium* Linn. revealed the presence of 16 putatively identified and characterized metabolites. Notable among these are 9,12-octadecadien-ol, n-hexadecanoic acid, octadecanoic acid, 2-oxo-, methyl ester in the Petroleum Ether extract; pentatriacontane, sulphurous acid, butyl heptadecyl ester, tritetracontane, heptacosane in the Chloroform extract; and tetrapentacontane, 1,54-dibromo,

docosane, 1,22-dibromo-triacontane, 1,30-dibromo, octadecanoic acid, 2-oxo-, methyl ester in the Ethyl Acetate extract; and 1-bromoeicosane, heneicosane, 11-cyclopentyl, 11-eicosenoic acid, trimethyl ester, 5-hydroxy-5-methyl-2-phenyl-2-oxazoline in the Ethanol extract.

A comprehensive presentation encompassing the active constituents, their respective retention times, molecular formulas, molecular weights, and percentage concentrations is provided. The abundance of these diverse bioactive metabolites within *E. foetidium* Linn. substantiates its traditional employment by practitioners for various maladies, while also indicating its potential for novel drug development, wherein individual constituents can be isolated and studied. A thorough exploration of the manifold substances found in *E. foetidium* Linn., as well as their prescription significance, is imperative, potentially paving the way for the synthesis of a multi-effect therapeutic agent in the foreseeable future.

CONCLUSION

The application of metabolomics through GC-MS serves to differentiate the source and offers an extensive spectrum of metabolites inherent to *E. foetidium* Linn. This encompasses a diverse range of organic acids, phenolic compounds, terpenoids, and alkaloids, all of which could potentially exert substantial influence within key metabolic pathways. Leveraging GC-MS analysis, we successfully identified and characterized a total of 17 secondary metabolites, all hitherto undiscovered in *E. foetidium* Linn. This novel revelation opens avenues for developing innovative therapeutic agents to combat and prevent diverse ailments in livestock. Isolating specific phytoconstituents for subsequent assessment of their biological efficacy emerges as a promising strategy. Undoubtedly, this approach holds immense promise for future drug development endeavors.

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